Reproductive Strategy and Cycle of the Toad-headed Agama *Phrynocephalus grumgrzimailoi* (Agamidae) in Xinjiang, China

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Abstract The reproductive strategy and cycle of *Phrynocephalus grumgrzimailoi* were studied at a locality close to Urumqi City, Xinjiang, China. The hatchlings of *P. grumgrzimailoi* need at least two years to reach sexual maturity, with the smallest mature male and female measured 48.02 mm and 47.01 mm snout-vent length (SVL), respectively. Adult females produce a single clutch per breeding season, with the clutch size ranging from 1 to 5. The clutch size and mass are significantly correlated with female SVL. There is no correlation of mean egg size with clutch size and relative fecundity in *P. grumgrzimailoi*, suggesting that the trade-off is absent between mean egg size and number. Females increase reproductive output mainly through increasing egg numbers. The copulation period lasts from April to June. Females begin vitellogenesis in April and lay eggs from May to July. Our results suggest that toad-headed lizards tend to select different reproductive strategies to adapt themselves to their arid or semi-arid habitats.

Keywords clutch size, relative fecundity, reproductive output, sexual maturity, trade-off

1. Introduction

Reproductive strategies and cycles are fundamental to evolutionary and behavioral studies. In this regard, squamate reptiles have been the focus of a relatively intensive study because, unlike many other vertebrates, they are often easily sampled and conveniently fed (Huey *et al.*, 2001; Olsson *et al.*, 2002; Vitt *et al.*, 2003). Plenty of research has focused on quantifying reproductive strategies of reptiles, with many studies describing clutch size variation (Kratochvíl and Frynta, 2006; Vitt and Price, 1982; Wang *et al.*, 2011), evolution of parental care (Shine, 1994), and reproductive seasonality (Vences *et al.*, 2004; Watling *et al.*, 2005; Zhao *et al.*, 2011). Links between reproductive ecology and life history represent trade-offs between size and number of offspring, costs of

producing offspring, seasonal production of offspring, and local environmental variation (Bertona and Chiaraviglio, 2003). Interactions among these factors determine the energy investment in reproduction, both in the short term for the individual's survival by balancing reproductive energy expenditure with daily maintenance energy requirements, and in the long term by determining an individual's success in transferring its genes into the next generation (Zug *et al.*, 2001).

Toad-headed agama lizards of the genus *Phrynocephalus* Kaup (Agamidae) are widely distributed in the deserts of arid or semi-arid regions in Central and West Asia, and northern and northwestern China (Zhao, 1997; Zhao *et al.*, 1999). Fourteen species in this genus are found in Xinjiang, China (Shi *et al.*, 2002).

Phrynocephalus grumgrzimailoi is the dominant species in eastern Xinjiang (Ananjeva *et al.*, 2011; Zhao *et al.*, 1999). *P. grumgrzimailoi* shows no sexual dimorphism in body size at subadult and adult stages. However, its males and females differ in head size and venter size at the subadult stage, with males having larger

head size and females having larger venter size compared to body size. This sexual dimorphism is more significant at mature stage (Liu and Shi, 2009). The objective of present study is to investigate the reproductive strategy and cycle of *P. grumgrzimailoi*.

2. Materials and Methods

2.1 Measurements of preserved specimens We examined specimens (deposited in the Museum of Xinjiang Agriculture University) that were collected close to Urumqi City (43.64° N, 87.59° E), Xinjiang, China from April to August in 1984. We measured snout-vent length (SVL, from the tip of snout to vent) and venter length (from axilla to groin) of 213 (male: 85; female: 128) preserved specimens. All measurements were made to the nearest 0.02 mm using vernier calipers. Sex of *P. grumgrzimailoi* was verified by dissection and direct examination of the gonads. Females were determined to be sexually matured when they contained oviductal eggs or enlarged vitellogenic follicles. Sexual maturity in males was determined by finding enlarged testes and convoluted epididymides. For active females in reproduction, we counted and measured the width and length (to the nearest 0.01 mm by digital vernier calipers) of the largest follicle or egg. Follicle or egg volume was estimated using the formula for a prolate spheroid (Vitt, 1991). For male, the length and width of the left testis was measured to the nearest 0.01 mm and estimated testis volume (Hibbitts *et al.*, 2005).

2.2 Measurements on collected gravid females 35 gravid females of *P. grumgrzimailoi* (verified by touch) were collected from the same place by hand or net from April to June in 2009, and then they were returned to the locality where they were caught after they ovulated. The lizards were housed in our laboratory in Urumqi, where females were marked by toe clipping for future identification. Mealworms (larvae of *Tenebrio molitor*) and water enriched with vitamins and minerals were provided. Eggs were collected, measured and weighed immediately after being produced. Postpartum females were measured and weighed; relative clutch mass (RCM) was calculated by dividing clutch mass by the postpartum female mass (Shine, 1992). The relative fecundity (RF) was defined as the residuals by regressing ln (clutch size) against ln (maternal SVL) (Olsson and Shine, 1997).

2.3 Data analysis We used SPSS (Statistical Product and Service Solutions) 11.5 for windows to analyze the data. All data were tested for normality (Kolmogorov-Smirnov Test) and homogeneity of variances (Levene's Test), and $\log_{10}$ transformation was performed when necessary to satisfy conditions for parametric tests. We used correlation analysis and post hoc tests to analyze the corresponding data. Throughout this paper, values are presented as mean ± standard error, and the significance level is set at α = 0.05.

2.4 Animal care Specimens were collected following the Guidelines for Use of Live Amphibians and Reptiles in Field Research. This work was carried out in compliance with the current laws on animal welfare and research in China.

3. Results

3.1 Reproductive measurements Descriptive statistics on reproductive indexes of *P. grumgrzimailoi* are given in Table 1. The largest female measured 61.21 mm SVL, with a minimum size at sexual maturity for females was recorded at 47.01 mm SVL. Clutch size (CS) of *P. grumgrzimailoi* averaged 3.23 ± 0.14 (n = 35), relative fecundity (RF) was 0.29 ± 0.01 (n = 35), mean egg mass (MEM) was 0.74 ± 0.02 g (n = 86), mean egg size (MES) was 661.69 ± 29.00 mm³ (n = 86), clutch mass (CM) was 2.5 ± 0.14 g (n = 27), and relative clutch mass (RCM) was 0.4 ± 0.02 (n = 27).

The CS and CM were significantly correlated with female SVL (CS: $r = 0.600$, $P < 0.001$, CM: $r = 0.790$, $P < 0.001$; Figure 1 A, B), while MEM and RCM showed no significant correlation with female SVL (MEM: $r = 0.375$, $P = 0.065$, RCM: $r = 0.383$, $P = 0.059$; Figure 1 C, D). The CS and CM were also significantly correlated with female venter length (CS: $r = 0.520$, $P = 0.007$, CM: $r = 0.710$, $P < 0.001$; Figure 2 A, B). However, there was no significant correlation between CS and MES ($r = 0.129$, $P = 0.531$; Figure 3 A), and MES was also independent

Table 1 Snout-vent length and reproductive characteristics of female *P. grumgrzimailoi*.

Index	n	Mean (SE)	Range
Snout-vent length (mm)	35	55.77 (0.57)	47.01–61.21
Post-oviposition body mass (g)	35	6.03 (0.27)	3.24–8.97
Clutch size	35	3.23 (0.14)	1–5
Relative fecundity	35	0.29 (0.01)	0.17–0.40
Mean egg mass (g)	86	0.74 (0.02)	0.53–1.09
Egg length (mm)	86	16.91 (0.16)	13.01–21.06
Egg width (mm)	86	8.54 (0.08)	6.59–9.9
Mean egg size (mm³)	86	661.69 (29.00)	371.07–1069.32
Clutch mass (g)	27	2.5 (0.14)	0.78–3.73
Relative clutch mass	27	0.4 (0.02)	0.21–0.55
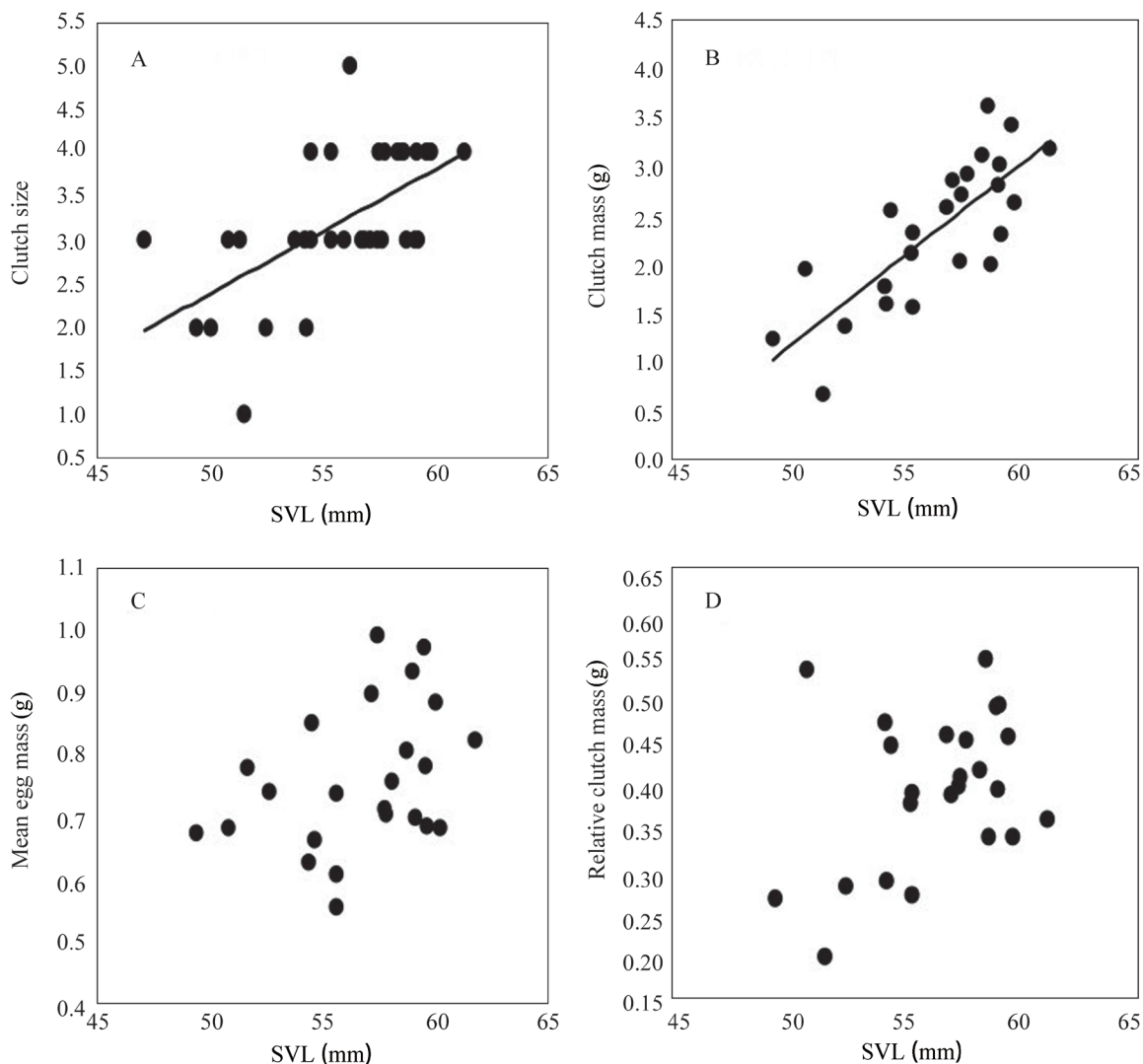
Figure 1 Relationships of clutch size (A), clutch mass (B), mean egg mass (C) and relative clutch mass (D) with snout-vent length of female in *P. grumgrzimailoi*. (C) and (D) do not show regression line because of no correlation of mean egg mass and relative clutch mass with SVL.

of RF ($r = 0.128$, $P = 0.532$; Figure 3 B), suggesting that the trade-off is absent in *P. grumgrzimailoi* between mean size and number of eggs.

3.2 Reproductive cycles Females larger than 47.01 mm SVL appeared to be at stages of reproduction, either with developing follicles or eggs (Figure 4 A), suggesting that females have the potential to reproduce after they reach this size, and follicles or eggs were at the same stage of development. Females ovulated under laboratory conditions from late May to late July. In preserved specimens, oviductal eggs were present from April to July, but absent from August to September (Figure 4 B), so parturition occurring in the field may be approximate to the time in the laboratory.

The smallest mature male was 48.02 mm SVL, with

males that were larger than this size displayed semi-turgid or turgid testes (Figure 5 A). After hibernation in April, the testes of adult males developed well, then the testes shrank significantly in June and July (post hoc tests: all $P < 0.01$), and before hibernation in September, their testes were significantly larger than those in all other months (post hoc tests: all $P < 0.001$) (Figure 5 B).

300 lizards of *P. grumgrzimailoi* less than 30 mm in SVL were collected during fieldwork after July in 2009, with the smallest individuals measuring 26.82 mm in SVL. The hatchlings (verified by navel) were returned to the locality where they were caught after being marked by toe clipping for future identification. Twelve of these lizards were recaptured the following year. Four individuals reached 48 mm SVL and attained sexual
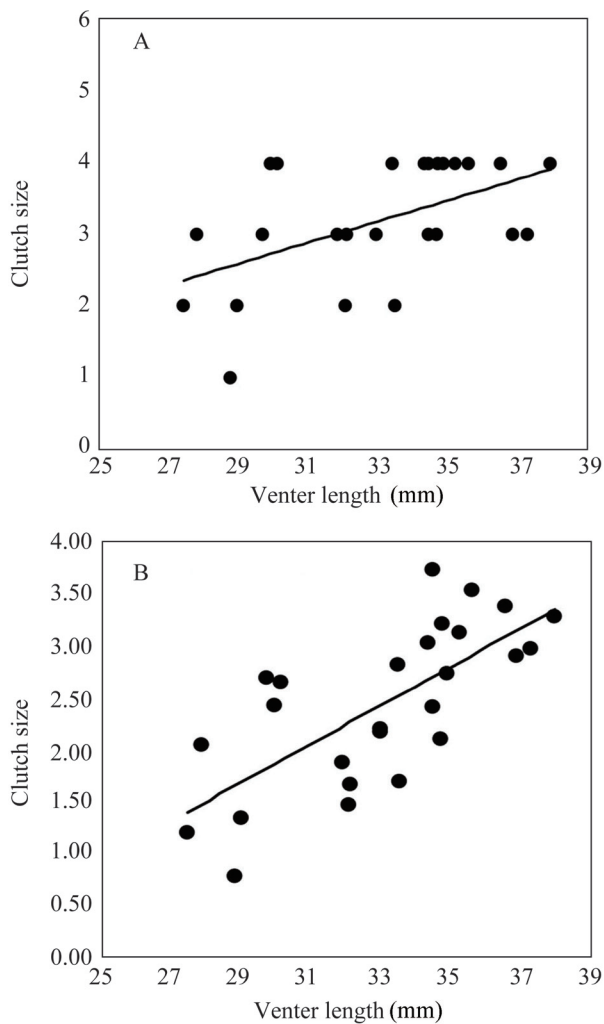
Figure 2 Relationships of clutch size (A) and clutch mass (B) with venter length of female in *P. grumgrzimailoi.*

maturity before hibernation, but could not breed because they had missed the breeding season. That is to say, 33% of juvenile *P. grumgrzimailoi* can be sexually mature the second year after birth.

4. Discussion

4.1 Reproductive strategy Reproductive output often covaries with female body size and/or shape in a variety of taxa including lizards (Ballinger, 1983; Goodman *et al.*, 2009). Our data show that maternal size and venter length are the main determinants of reproductive output in *P. grumgrzimailoi*. Females with larger size and longer venter produce more eggs, and thus, heavier clutches (Figure 1 A, B, Figure 2 A, B), indicating that the females of *P. grumgrzimailoi* increase reproductive output mainly through increasing egg numbers. However, mean egg size was independent of clutch size and relative fecundity,

suggesting that the predicted trade-off (an inverse relationship) between mean egg size and number of eggs was not found in this study. The size of a hatchling depends on egg size (Braña and Ji, 2000; Sinervo, 1990) and egg size should vary with maternal size and/ or clutch size in species where eggs are not optimized for size (Doughty and Shine, 1997; Olsson and Shine, 1997), which means that our results, although not completely conclusive because other untested potential causes, might contribute to the variation in egg size and show that the females of *P. grumgrzimailoi* tend to produce optimally sized egg.

Our results are different from those obtained from some other oviparous toad-headed lizards (Qu *et al.*, 2011), but similar to those from viviparous *P. vlangali* (Zhang *et al.*, 2005). This evidence shows that the females of *P. grumgrzimailoi* tend to select different reproductive
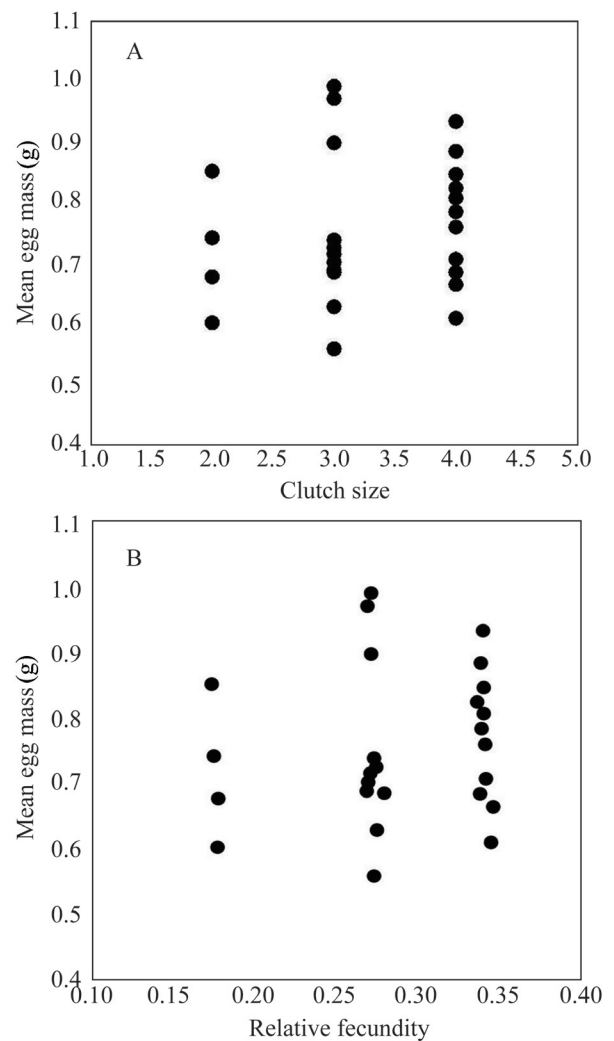
Figure 3 Relationships of mean egg size to clutch size (A) and relative fecundity (B) of female in *P. grumgrzimailoi*. Regression line is not shown because no correlation exists.
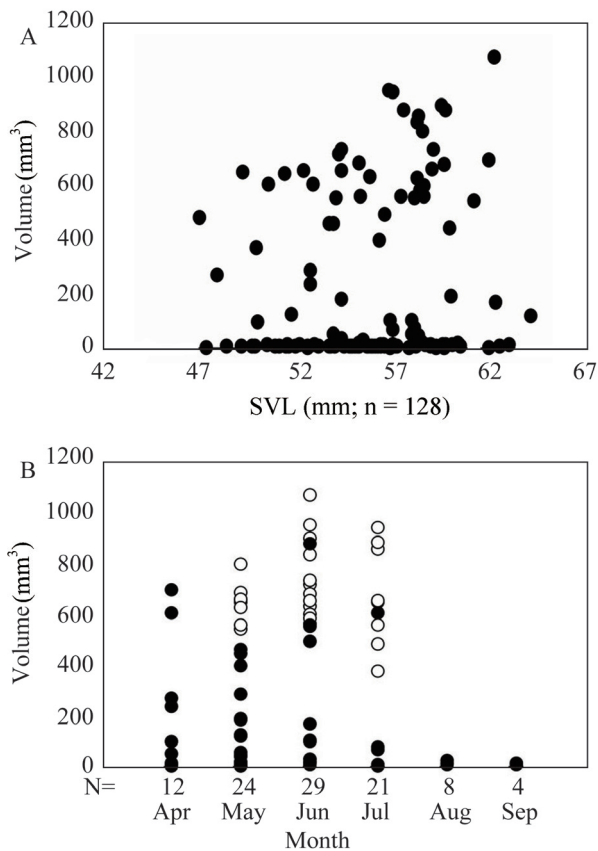
Figure 4 Estimated volumes of individual eggs in relation to SVL (A) and month (B) of female in *P. grumgrzimailoi*. Solid dots: Oviductal eggs or enlarged vitellogenic follicles; Open dots: Eggs.

strategies to adapt themselves to their arid or semi-arid habitats.

4.2 Reproductive cycle Most lizards from the environments with predictable climate conditions in the temperate zone and seasonal tropics usually have obvious breeding cycles such as courtship, mating and incubation occurring at certain times during the year (Al-Johany *et al.*, 1997; Castilla and Bauwens, 1990; Vences *et al.*, 2004; Vitt and Caldwell, 2009), whereas reproduction of lizard species from tropical regions without seasonal fluctuation usually lack reproductive peaks at the population level, with reproductive activities of males and females occurring throughout the year (Ramírez-Sandoval *et al.*, 2006; Zug *et al.*, 2001). In this study, specimens developed follicles or eggs from April to July, while they had no vitellogenic follicles in August. Based on variation in the size of vitellogenic follicles, we can speculate on the times and period of oviposition (Aldridge and Semlitsch, 1992). Our results indicate that the females of *P. grumgrzimailoi* had a reproductive cycle similar to that of the other temperate lizards, that is, ovulation occurs in late spring, and eggs are laid in summer (James

and Shine, 1985). This evidence is usually used as an indicator of one clutch per year. Several studies on other Chinese *Phrynocephalus* species of both in alpine (Zhang *et al.*, 2005) and in desert (Chen *et al.*, 1993; Liu *et al.*, 1996; Qu *et al.*, 2011) also produced one clutch per year except *P. przewalskii* (Zhao *et al.*, 2011).

Based on the variation in testis size, we can speculate on the period of copulation (Aldridge, 1993). The adult males after hibernation were found with their testes fairly developed, while they were inactive in July, and before hibernation with testis volume reached the maximum.

Our result shows that the copulation period of *P. grumgrzimailoi* lasts from post-hibernation to June. This evidence is similar to other toad-headed agama lizards of the genus *Phrynocephalus* (Chen *et al.*, 1993; Song *et al.*, 1987). Spermatogenetic cycles usually coincide with ovarian cycles in temperate reptiles, and the male cycle in these species is considered prenuptial because mating takes place prior to the production of eggs (Vitt and Caldwell, 2009).
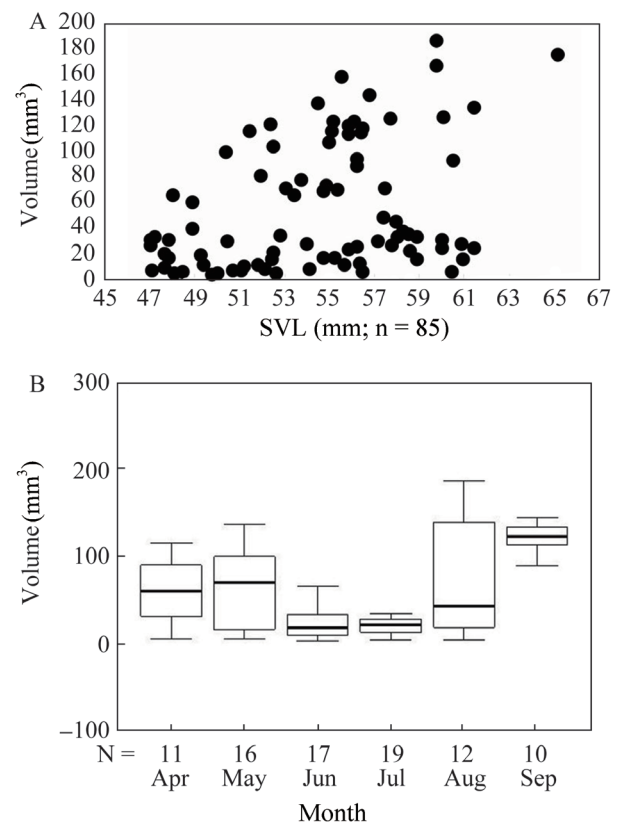
Figure 5 Estimated testis volumes in relation to SVL (A) and month (B) of male in *P. grumgrzimailoi*. Box quartile plot for estimated testis volumes. The box represents the quartiles, the extensions represent the data range, and the solid line represents the mean.

References

Al-Johany A. M., Al-Sadoon M. K., Al-Farraj S. A. 1997. Reproductive biology of the skink *Scincus mitranus* (Anderson, 1871) in the central region of Saudi Arabia. J Arid Environ, 36: 319–326

Aldridge R. D., Semlitsch R. D. 1992. Female reproductive biology of the southeastern crowned snake (*Tantilla coronata*). Amphibia-Reptilia, 13: 209–218

Aldridge R. D. 1993. Male reproductive anatomy and seasonal occurrence of mating and combat behavior of the rattlesnake *Crotalus v. viridis*. J Herpetol, 27: 481–484

Ananjeva N. B., Guo X. G., Wang Y. Z. 2011. Taxonomic diversity of agamid lizards (Reptilia, Sauria, Acrodonta, Agamidae) from China: A comparative analysis. Asian Herpetol Res, 2(3): 117–128

Ballinger R. E. 1983. Life-history variations. In Huey R. B., Pianka E. R., Schoener T. W. (Eds.), Lizard Ecology: Study of a Model Organism. Cambridge, UK: Harvard University Press, 241–260

Bertona M., Chiaraviglio M. 2003. Reproductive biology, mating aggregations, and sexual dimorphism of the Argentine boa constrictor (*Boa constrictor occidentalis*). J Herpetol, 37: 510–516

Braña F., Ji X. 2000. Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards (*Podarcis muralis*). J Exp Zool, 286: 422–433

Castilla A. M., Bauwens D. 1990. Reproductive and fat body cycles of the lizard, *Lacerta lepida*, in central Spain. J Herpetol, 24: 261–266

Chen Q., Han Z. X., Song Z. M. 1993. A study on the reproduction of lizard *Phrynocephalus versicolor*. J Lanzhou Univ (Nat Sci), 29: 199–203 (In Chinese)

Doughty P., Shine R. 1997. Detecting life history trade-offs: measuring energy stores in "capital" breeders reveals costs of reproduction. Oecologia, 110: 508–513

Goodman B. A., Hudson S. C., Isaac J. L., Schwarzkopf L. 2009. The evolution of body shape in response to habitat: is reproductive output reduced in flat lizards? Evolution, 63: 1279–1291

Hibbitts T. J., Pianka E. R., Huey R. B., Whiting M. J. 2005. Ecology of the common barking gecko (*Ptenopus garrulus*) in southern Africa. J Herpetol, 39: 509–515

Huey R. B., Pianka E. R., Vitt L. J. 2001. How often do lizards "run on empty"? Ecology, 82: 1–7

James C., Shine R. 1985. The seasonal timing of reproduction. Oecologia, 67: 464–474

Kratochvíl L., Frynta D. 2006. Egg shape and size allometry in geckos (Squamata: Gekkota), lizards with contrasting eggshell structure: Why lay spherical eggs? J Zool Syst Evol Res, 44: 217–222

Liu N. F., Chen Q., Xie X. M. 1996. Reproductive ecology of *Phrynocephalus pezewalskii*. Acta Ecol Sin, 16: 276–282 (In Chinese)

Liu Y., Shi L. 2009. Ontogenetic shifts of sexual dimorphism in *Phrynocephalus grumgrzimailoi* (Agamidae). Sichuan J Zool, 28: 710–713 (In Chinese)

Olsson M., Shine R. 1997. The limits to reproductive output: offspring size versus number in the sand lizard (*Lacerta agilis*). Am Nat, 149: 179–188

Olsson M., Shine R., Wapstra E., Ujvari B., Madsen T. 2002. Sexual dimorphism in lizard body shape: The roles of sexual selection and fecundity selection. Evolution, 56: 1538–1542

Qu Y. F., Gao J. F., Mao L. X., Ji X. 2011. Sexual dimorphism and female reproduction in two sympatric toad-headed lizards, *Phrynocephalus frontalis* and *P. versicolor* (Agamidae). Anim Biol, 61: 139–151

Ramírez-Sandoval E., Ramírez-Bautista A., Vitt L. J. 2006. Reproduction in the lizard *Phyllodactylus lanei* (Squamata: Gekkonidae) from the Pacific Coast of Mexico. Copeia, 2006(1): 1–9

Shi L., Zhou Y. H., Yuan H. 2002. Reptile fauna and geographic divisions in Xinjiang Uygur Autonomous Region. Sichuan J Zool, 21: 152–156 (In Chinese)

Shine R. 1992. Relative clutch mass and body shape in lizards and snakes: Is reproductive investment constrained or optimized? Evolution, 46: 828–833

Shine R. 1994. Sexual size dimorphism in snakes revisited. Copeia, 1994: 326–346

Sinervo B. 1990. The evolution of maternal investment in lizards: An experimental and comparative constrained or optimized. Evolution, 46: 828–833

Song Z. M., Chen L., Chen Q. 1987. A study on the reproduction of *Phrynocephalus przewalskii*. Chin Herpetol Sin, 6(1): 12–17 (In Chinese)

Vences M., Galán P., Miramontes K., Vieites D. R. 2004. Weak expression of reproductive seasonality in a dwarf gecko (*Lygodactylus verticillatus*) from arid south-western Madagascar. J Arid Environ, 56: 329–338

Vitt L. J., Price H. J. 1982. Ecological and evolutionary determinants of relative clutch mass in lizards. Herpetologica, 38: 237–255

Vitt L. J. 1991. Ecology and life history of the scansorial arboreal lizard *Plica plica* (Iguanidae) in Amazonian Brazil. Can J Zool, 69: 504–511

Vitt L. J., Pianka E. R., Cooper W. E., Schwenk K. 2003. History and the global ecology of squamate reptiles. Am Nat, 162: 44–60

Vitt L. J., Caldwell J. P. 2009. Herpetology: An Introductory Biology of Amphibians and Reptiles. 3rd Ed. Oxford, UK: Academic Press

Wang Z., Xia Y., Ji X. 2011. Clutch frequency affects the offspring size-number trade-off in lizards. PloS ONE, 6: e16585

Watling J. I., Waddle J. H., Kizirian D., Donnelly M. A. 2005.

Reproductive phenology of three lizard species in Costa Rica, with comments on seasonal reproduction of neotropical lizards. J Herpetol, 39: 341–348

Zhang X. D., Ji X., Luo L. G., Gao J. F., Zhang L. 2005. Sexual dimorphism and female reproduction in the Qinghai toad-headed lizard *Phrynocephalus vlangalii*. Acta Zool Sin, 51: 1006–1012

Zhao E. M., Zhao K. T., Zhou K. Y. 1999. Fauna Sinica, Reptilia Vol. 2, Squamata, Lacertilia. Beijing: Science Press (In Chinese)

Zhao K. T. 1997. Toad-headed agamids in China. Chin J Zool, 32: 15–19 (In Chinese)

Zhao W., Yu N. N., Wang Y. J., Ji W. H., Liu N. F. 2011. Female reproductive cycles of *Phrynocephalus przewalskii* (Lacertilia: Agamidae) in the Tengger Desert, China. Asian Herpetol Res, 2(1): 30–35

Zug G. R., Vitt L. J., Caldwell J. P. 2001. Herpetology: An Introductory Biology of Amphibians and Reptiles. 2nd Ed. San Diego, USA: Academic Press